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Checkpoint

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13. ABSTRACT (Maximum 200 Words)

The ATR-dependent DNA damage response pathway can respond to a diverse group of lesions as well as inhibitors of DNA replication. Using the Xenopus egg extract system, we show that lesions induced by UV irradiation and cis-platinum cause the functional uncoupling of MCM helicase and DNA polymerase activities, an event previously shown for aphidicolin. Inhibition of uncoupling during elongation with inhibitors of MCM7 or Cdc45, a putative helicase cofactor, results in abrogation of Chk1 phosphorylation, indicating that uncoupling is necessary for activation of the checkpoint. However, uncoupling is not sufficient for checkpoint activation, and DNA synthesis by Polα is also required. Finally, using plasmids of varying size, we demonstrate that all of the unwound DNA generated at a stalled replication fork can contribute to the level of Chk1 phosphorylation, suggesting that uncoupling amplifies checkpoint signaling at each individual replication fork. Taken together, these observations indicate that functional uncoupling of MCM helicase and DNA polymerase activities occurs in response to multiple forms of DNA damage and that there is a general mechanism for generation of the checkpoint-activating signal following DNA damage.

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Introduction

The DNA damage checkpoint pathway is a highly conserved surveillance mechanism that senses the presence of DNA damage and elicits an immediate and effective response to genotoxic insults. This pathway is required to maintain genomic integrity. The focus of our research has been in elucidating the mechanism by which this pathway senses DNA damage. Previously, we had shown that activation of this pathway requires initiation of DNA replication. In the last year, we have identified the replication-dependent step in checkpoint activation. In this report, we describe out results demonstrating that it is the imbalance in the rates of DNA unwinding and DNA synthesis which is responsible for activation of this pathway.

Body:

The following results are in Press in Genes and Development.

ATR, Rad1 and Claspin-Mediated Checkpoint Activation with Plasmid DNA.

We and others have previously shown that addition of aphidicolin, MMS, and UV-treated chromatin to *Xenopus* egg extracts leads to replication-dependent accumulation of RPA on chromatin (Michael et al. 2000; Mimura et al. 2000; Walter 2000; Lupardus et al. 2002). This observation indicates that ssDNA accumulates following these forms of DNA damage and that some replication-dependent event is necessary for that accumulation. Using the *Xenopus* egg extract system, we have also shown that aphidicolin treatment can lead to the formation of a highly unwound form of plasmid DNA, suggesting that the activity of the helicase can become uncoupled from the DNA polymerase (Walter and Newport 2000). We wanted to determine if the topological changes that occur in plasmid DNA following aphidicolin treatment are coupled to checkpoint activation. Thus, we first tested the idea that the ATR-mediated checkpoint could be studied using plasmid DNA. For these studies we used a completely soluble system derived from *Xenopus* egg extracts that allows replication of plasmid DNA (Walter et al., 1998). Efficient replication of plasmid DNA or chromatin in this system requires the initial incubation of the DNA in cytosol to assemble the pre-replicative complex (pre-RC). Subsequent addition of a concentrated nucleoplasmic extract (NPE) supplies high levels of cdk2 and cdc7 kinase activities, thereby allowing initiation of DNA replication (Walter 2000; Prokhorova et al. 2003).

When plasmid DNA was incubated with cytosol and then supplemented with NPE containing aphidicolin, Chk1 underwent robust phosphorylation on S344 (Figure 1A, lane 3). This residue is phosphorylated in an ATR-dependent manner following UV and aphidicolin treatment (Guo et al. 2000; Hekmat-Nejad et al. 2000; Liu et al. 2000).

Incubation of UV-damaged plasmid DNA in cytosol followed by NPE also led to phosphorylation of Chk1 on the same residue (Figure 1A, lane 5). Inhibition of replication with geminin (McGarry and Kirschner 1998) or p27^{KIP} (Walter and Newport 2000) resulted in a complete loss of Chk1 phosphorylation following both UV and aphidicolin treatment, indicating that both responses are replication-dependent (Figure 1A, lanes 4,6,8-9).

To determine if the ATR complex, RHR complex and Claspin are required for Chk1 phosphorylation in this plasmid-based system, as they are for chromatin, we immunodepleted ATRIP, Rad1, and Claspin from both cytosol and NPE (Figures 1B & 1C). Although aphidicolin induced robust Chk1 phosphorylation in mock-depleted extracts, no detectable Chk1 phosphorylation was observed using extracts immunodepleted of ATRIP, Rad1 or Claspin (Figures 1D & 1E). Moreover, addition of recombinant Claspin to Claspin-depleted extracts restored Chk1 phosphorylation (Figure 1E). These observations demonstrate that the ATR-ATRIP complex, the RHR complex and Claspin mediate the checkpoint induced by aphidicolin during replication of plasmid DNA.

Chk1 Activation Follows Aphidicolin-Induced DNA Unwinding. To examine the relationship between DNA unwinding and checkpoint activation, we monitored the topology of plasmid DNA on chloroquine gels in the presence of aphidicolin. Upon protein extraction, plasmids that have been extensively unwound by a DNA helicase are rendered highly negatively supercoiled. Thus, they migrate rapidly on agarose gels even in the presence of high concentrations of chloroquine, which unwinds DNA upon intercalation and causes compensatory positive supercoiling in closed circular plasmids (Walter and Newport 2000). We found that this hyperunwound form of DNA (U form)

was generated in a reversible manner at concentrations of aphidicolin as low as 1.3 µM

and that increasing the concentration of aphidicolin led to a dose-dependent persistence

of this unwound form. Aphidicolin was added to the cytosol prior to the initiation of

replication triggered by NPE addition. To determine whether the appearance and

disappearance of U form DNA correlated with activation of Chk1, we examined the

relationship between the phosphorylation state of Chk1 and the topology of DNA at

these different concentrations of aphidicolin. The phosphorylation of Chk1 occurred

within 10 minutes of the appearance of U form DNA and was stronger at higher doses of

aphidicolin (Figure 2). Furthermore, the phosphorylation of Chk1 persisted as long as U

form DNA was present, and Chk1 dephosphorylation followed the disappearance of U

form DNA. These observations indicate that there is a temporal relationship between

the unwinding of DNA induced by aphidicolin and Chk1 phosphorylation. They are also

consistent with a previous study demonstrating a correlation between RPA accumulation

on chromatin and Chk1 phosphorylation (Shechter et al. 2004a).

Replication of Damaged Plasmid DNA Leads to Uncoupling of Helicase and

Polymerase Activities. We previously showed that RPA accumulates on chromatin

following UV irradiation and MMS treatment (Lupardus et al. 2002). One possibility is

that this accumulation is due to the binding of RPA to ssDNA generated during repair of

the lesions. However, it is also possible that these lesions cause accumulation of

ssDNA by inducing uncoupling of helicase and polymerase activities, as observed for

aphidicolin (Walter and Newport 2000). To distinguish between these possibilities, we

asked whether U form DNA was formed upon replication of plasmid DNA damaged by

UV irradiation. We observed a dose-dependent formation of U form DNA upon UV

treatment (Figure 3A). Inhibition of origin firing with geminin completely abrogated the

accumulation of U form in response to UV damage (data not shown). Importantly, the disappearance of U form DNA correlated with increased DNA synthesis, as monitored by radiolabeled dCTP incorporation (Figure 3A). This indicates that loss of U form DNA observed at 500 and 1000 J/m^2 can be attributed to DNA replication on the unwound strand. Significantly, when the phosphorylation state of Chk1 at S344 was examined in parallel, we found that the phosphorylation of Chk1 consistently followed the appearance of U form DNA (Figure 3A). These observations indicate that UV irradiation, like aphidicolin, leads to hyperunwinding of DNA and are consistent with a role for DNA hyperunwinding in activation of the DNA damage checkpoint.

To determine if other forms of DNA damage can also induce the functional uncoupling of helicase and polymerase activities, we examined the effect of the chemotherapeutic cis-platinum. Cis-platinum has been shown to induce ATR-dependent cell cycle arrest and Chk1 phosphorylation (Cliby et al. 1998; Zhao and Piwnica-Worms 2001). First, we examined whether lesions generated by cis-platinum treatment induced the functional uncoupling of helicase and polymerase activities. Upon replication of plasmid DNA damaged with cis-platinum, significant accumulation of U form DNA was observed (Figure 3B). Moreover, when the initiation of replication was blocked with geminin, the formation of U form DNA was abrogated. No detectable level of U form was observed in samples containing mock-treated plasmid DNA. When we examined the phosphorylation state of Chk1 on S344 in parallel, we observed significant checkpoint activation upon replication of cis-platinum-treated plasmid DNA. No detectable level of Chk1 phosphorylation was observed upon replication of the mock-treated plasmid. It is notable that cis-platinum forms both intra- and inter-strand crosslinks, the latter of which might be expected to block unwinding and checkpoint activation. However, the vast majority of lesions caused by cis-platinum are intra-strand

crosslinks (Kartalou and Essigmann 2001), and we expect that the predominance of these intra-strand lesions allows for sufficient uncoupling to induce the checkpoint. Taken together, these results show for the first time that two DNA damaging agents that induce ATR activation, UV and cis-platinum, cause the uncoupling of helicase and polymerase activities that leads to DNA hyperunwinding.

Inhibition of MCM-Mediated Hyperunwinding During Elongation Blocks Chk1 Phosphorylation. The relationship between U form DNA and Chk1 phosphorylation suggests that hyperunwinding may be required for checkpoint activation. Because aphidicolin, UV and cis-platinum-induced hyperunwinding are dependent on the initiation of DNA replication ((Walter and Newport 2000) and data not shown), they may reflect uncoupling of the replicative DNA helicase from the stalled polymerase. We, and others, have recently shown that the MCM2-7 complex (Pacek and Walter 2004; Shechter et al. 2004b), as well as the replication factor Cdc45 (Pacek and Walter 2004), are essential for unwinding during both the beginning and middle of S phase. These results strongly support the hypothesis that MCM2-7 functions as the replicative DNA helicase (Labib and Diffley 2001), and that Cdc45 acts as a helicase co-factor (Masuda et al. 2003).

To directly test the hypothesis that MCM-mediated hyperunwinding is required to launch the checkpoint, we allowed replication to initiate, and then stalled the elongating complex with aphidicolin in the presence and absence of helicase inhibitors. Briefly, chromatin was incubated in cytosol to allow pre-RC formation, and it was then mixed with NPE for 25 minutes at a reduced temperature (19°C) to allow initiation of DNA replication but not completion of elongation (Figure 4A). Although our previous data

indicate that all origins fired during the low temperature incubation (Pacek and Walter 2004), p27^{KIP} was added here and in all subsequent steps to insure that no additional origins could fire. The partially replicated chromatin was isolated and incubated with buffer, purified Cdc45-neutralizing antibodies or antibodies pre-mixed with recombinant Cdc45 protein. The treated chromatin samples were then added to NPE containing aphidicolin. Significant accumulation of RPA on chromatin and caffeine-sensitive Chk1 phosphorylation on S344 were observed with the buffer-treated chromatin when aphidicolin was present, indicating that hyperunwinding and checkpoint activation had occurred (Figure 4A, lanes 2 and 3). Importantly, treatment of the isolated chromatin with Cdc45 neutralizing antibodies significantly reduced RPA accumulation and Chk1 phosphorylation (Figure 4A, lane 4). However, pre-incubation of the neutralizing antibodies with recombinant Cdc45 protein restored RPA accumulation and Chk1 phosphorylation (Figure 4A, lane 5). The loading of MCM7, ORC2 and Cdc45 onto chromatin was unaffected by any of these treatments. These observations show for the first time that Cdc45-dependent hyperunwinding during elongation is necessary for activation of Chk1 following aphidicolin treatment.

As an independent means of blocking hyperunwinding, we inhibited the MCM2-7 complex. For these experiments we used an N-terminal fragment of the retinoblastoma protein (Rb$^{1-400}$) that binds to MCM7 and inhibits DNA replication and aphidicolin-induced unwinding (Sterner et al. 1998; Pacek and Walter 2004). DNA replication forks were synchronized during the elongation phase of DNA replication as described above. Chromatin was isolated and mixed with buffer, Rb$^{1-400}$ or Rb$^{1-400}$ that had been pre-incubated with MCM7 peptide. Finally, NPE-containing aphidicolin was added. As seen in Figure 4B, aphidicolin induced RPA hyperloading (compare lanes 1 and 2), and caffeine-sensitive Chk1 phosphorylation (compare lanes 2 and 3). Importantly, Rb$^{1-400}$

inhibited both RPA hyperloading and Chk1 phosphorylation (Figure 4B, lane 4).

Inhibition was reversed when Rb$^{1-400}$ was pre-incubated with MCM7 peptide (Figure 4B,

lane 5). Taken together, these observations strongly suggest that in the context of a

stalled DNA replication fork, MCM and Cdc45-dependent DNA hyperunwinding is

necessary for the phosphorylation and activation of Chk1.

The Amount of DNA Unwinding Determines the Level of Chk1 Activation

Induced with Aphidicolin. We previously showed that replication of a 3-kb plasmid in

the presence of aphidicolin results in extensive unwinding (Walter and Newport 2000).

Although we show that functional uncoupling of DNA unwinding and DNA synthesis is

required for checkpoint activation, it is not known if the entire region of hyperunwound

DNA contributes to checkpoint signaling. In fact, previous observations in *S. cerevisiae*

suggest that extensive unwinding may not occur. Specifically, EM studies indicate that

the amount of additional ssDNA generated at replication forks stalled with HU is

approximately 100 nucleotides (Sogo et al. 2002). Consistent with this, CHIP studies

show also that uncoupling of Cdc45 and RPA from DNA synthesis is minimal in wild-type

yeast cells treated with HU (Katou et al. 2003). In addition, it has been suggested that

the number of functional replication forks may determine the level of checkpoint

activation in *S. cerevisiae* (Shimada et al. 2002).

We sought to test the contribution of the unwound DNA to Chk1 phosphorylation

in the *Xenopus* system. If the number of origins, and thus the number of replication

forks, determines the level of checkpoint activation, comparable levels of Chk1

phosphorylation should be observed with aphidicolin when equal *molar* quantities of

different sized plasmids are examined, assuming an equal number of origins per

plasmid. On the other hand, if the unwound DNA contributes to checkpoint activation,

then larger plasmids should produce a greater signal with aphidicolin when added on an equal *molar* basis since these plasmids should have more unwound DNA per mole of plasmid.

To determine whether the unwound DNA contributes to Chk1 phosphorylation, we tested three different sized plasmids and compared the levels of Chk1 phosphorylation generated upon aphidicolin treatment. Plasmids of less than 10-kB are replicated via a single origin in *Xenopus* egg extracts (Lucas et al. 2000). Therefore, we initially used equal *molar* amounts of a 0.8-kb, 3.0-kb and 9-kb plasmid in these experiments. The 0.8-kb plasmid was generated by intramolecular self-ligation of a blunt-ended PCR product, and the plasmid was shown to replicate when incubated sequentially in cytosol then NPE (data not shown). In addition, this plasmid induced Chk1 phosphorylation on S344 following aphidicolin treatment in a geminin-sensitive manner (data not shown).

We found that the level of Chk1 phosphorylation increased with increasing plasmid size when the plasmids were present on an equal *molar* basis (Figure 5A). Interestingly, we also found that the amount of Chk1 phosphorylation was roughly proportional to the size of the plasmid (Figure 5A). Consistent with this, when the 9-kb and 0.8-kb plasmids were present on an equal *mass* basis, the levels of Chk1 phosphorylation observed were comparable (Figure 5B). These data strongly suggest that it is not the number of origins or replication forks that determines the level of checkpoint activation, but rather the amount of unwound DNA. They also demonstrate that 800 bp of unwound DNA is sufficient to induce the phosphorylation of Chk1 and that additional unwinding contributes to the extent to which Chk1 is phosphorylated.

Uncoupling of MCM Helicase and DNA Polymerase Activities is Not Sufficient For Checkpoint Activation. Although we observed a temporal relationship between U form DNA and checkpoint activation, Chk1 phosphorylation consistently lagged behind U form DNA by at least 10 minutes with both UV and aphidicolin (Figures 2 and 3). This indicates that DNA unwinding may be necessary, but not sufficient, to trigger the checkpoint. Since this effect appeared to be more pronounced at higher UV and aphidicolin doses, we examined the effect of further increasing the concentration of aphidicolin on checkpoint activation during replication of plasmid DNA. The addition of 15 μM aphidicolin induced robust Chk1 phosphorylation by the 30 minute time point (Figure 6A). However, when aphidicolin was present at 150 or 375 μM, checkpoint activation was significantly delayed, and at a concentration of 1.1 mM, Chk1 phosphorylation was undetectable. Plasmid hyperunwinding was observed at all concentrations of aphidicolin tested, as determined by the generation of U form DNA, but replication was progressively inhibited as the concentration of aphidicolin was increased. The appearance of U form DNA at high concentrations of aphidicolin indicates that hyperunwinding is not sufficient for activation of Chk1 (Figure 6A). Importantly, however, the phosphorylation of Chk1 strongly correlated with the extent of DNA replication, suggesting that the synthesis of DNA is necessary for checkpoint activation. Moreover, when 1.5 mM aphidicolin was added during S-phase, we observed robust checkpoint activation (Figure 6B). This latter observation demonstrates that checkpoint activation can occur with 1.5 mM aphidicolin if some DNA synthesis has already occurred.

As an alternative method of inhibiting DNA synthesis, we examined the effects of inhibiting Polα with the monoclonal antibody SJK-132. SJK-132 has been shown to specifically inhibit the DNA polymerase activity of Polα (Tanaka et al. 1982) and induce

the phosphorylation of Chk1 in *Xenopus* egg extracts (Michael et al. 2000). If DNA

synthesis is required for checkpoint activation, we would expect that, like aphidicolin,

partial disruption of DNA replication with SJK-132 would activate the checkpoint by

inducing an uncoupling event and allowing an adequate level of DNA synthesis.

However, further inhibition would eventually result in loss of the checkpoint, as observed

for aphidicolin (Figure 6A). Consistent with this hypothesis, the addition of SJK-132 to a

final concentration of 44 μg/ml or 176 μg/ml induced significant Chk1 phosphorylation

(Figure 6C). However, upon addition of SJK-132 to a final concentration of 880 μg/ml, a

dramatic decrease in the level of Chk1 phosphorylation was observed (Figure 6C). The

addition of SJK-132 to a final concentration of 880 μg/ml in crude interphase extracts

also abrogated the Chk1 phosphoryation that occurs in response to aphidicolin and UV

damaged chromatin (data not shown). Importantly, significant RPA accumulation on

chromatin was observed at all the concentrations of SJK-132 examined, demonstrating

that the loss of the checkpoint did not result from a lack of nuclear envelope formation,

origin firing or a failure to functionally uncouple helicase and polymerase activities (data

not shown). Taken together, these data demonstrate a requirement for DNA polymerase

alpha in activation of the ATR-dependent checkpoint, consistent with previous

observations (Michael et al. 2000). However, since aphidicolin and SJK-132 inhibit DNA

polymerase activity and not RNA primer synthesis (Yagura et al. 1983; Sheaff et al.

1991), these results further suggest it is the DNA polymerase activity of Polα that is

critical for checkpoint activation.

DNA Polymerase Activity of Polα is Needed for Rad1 Chromatin Binding.

We had previously shown that aphidicolin induces the recruitment of the ATR and Rad1

checkpoint complexes onto chromatin in egg extracts (Lupardus et al. 2002). In order to

determine how aphidicolin acts to block checkpoint activation at high concentrations

(Figure 6A), we examined the chromatin binding of both checkpoint protein complexes.

When 25 μM aphidicolin was added to extracts, robust Chk1 phosphorylation was

observed, and RPA, Polα, PCNA, ATRIP, Claspin, and Rad1 accumulated on chromatin

(Figure 7). However, when the concentration of aphidicolin was increased to 740 μM,

Chk1 phosphorylation and chromatin binding of Rad1 was abrogated. This

concentration of aphidicolin also blocks PCNA loading (Figure 7 and (Michael et al.

2000; Arias and Walter 2005)), by preventing adequate elongation of the RNA/DNA

primer synthesized by Polα. Importantly, the loading of RPA, Polα, Claspin, and ATRIP

were unaffected (Figure 7). Taken together, these observations suggest that recruitment

of RPA, ATRIP and Polα is not sufficient for Rad1 chromatin binding or Chk1

phosphorylation and that synthesis and elongation of a DNA primer is necessary to

recruit the RHR complex onto chromatin for activation of Chk1.
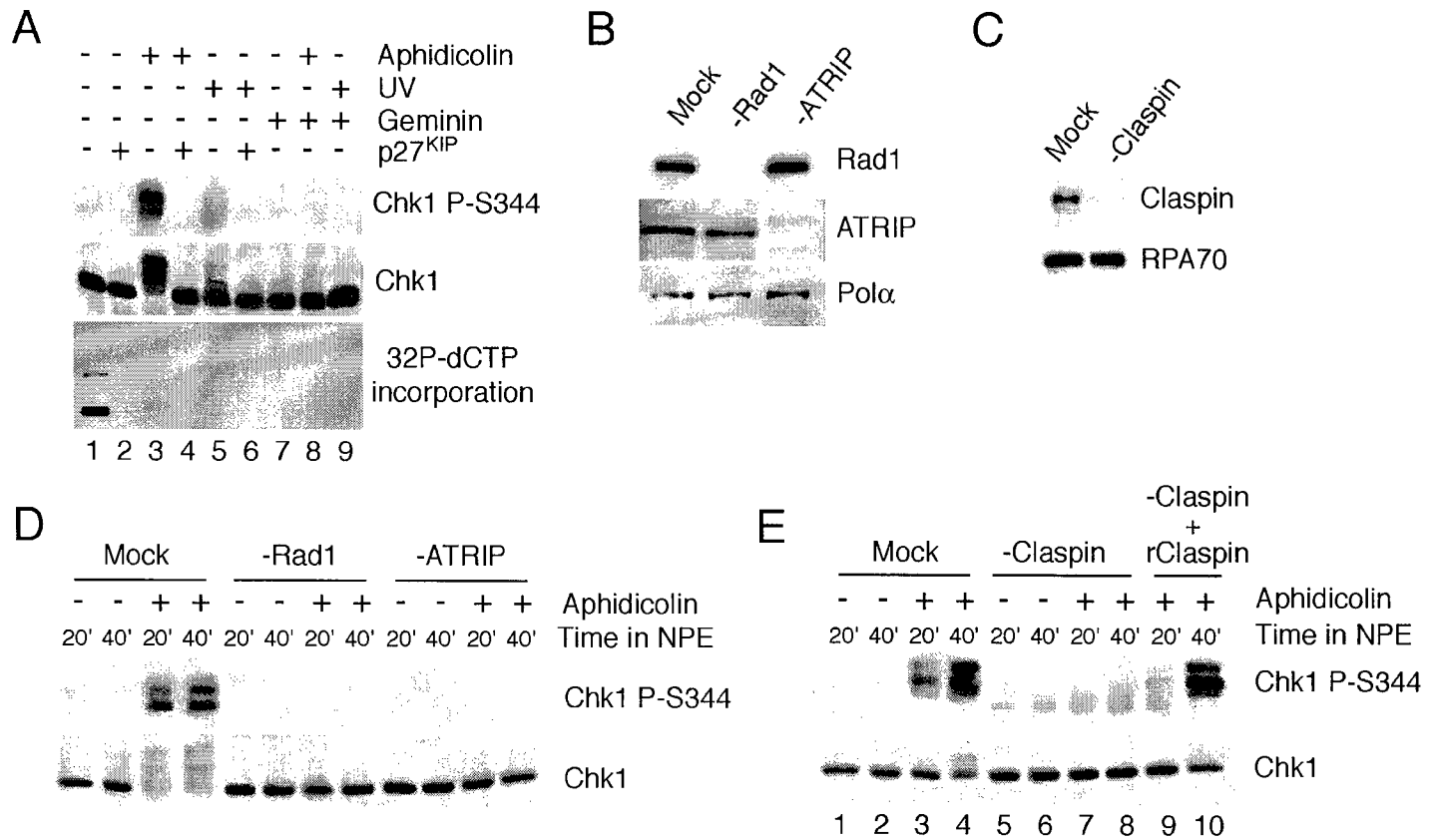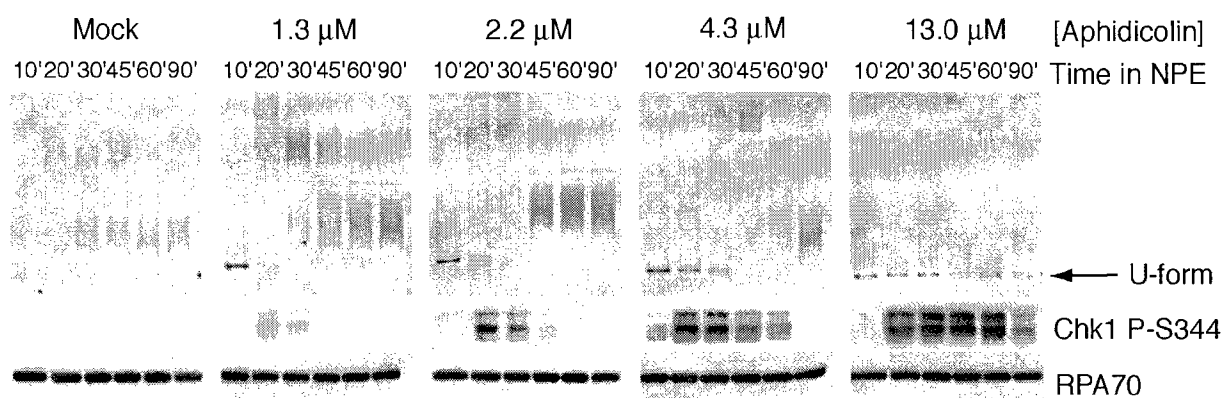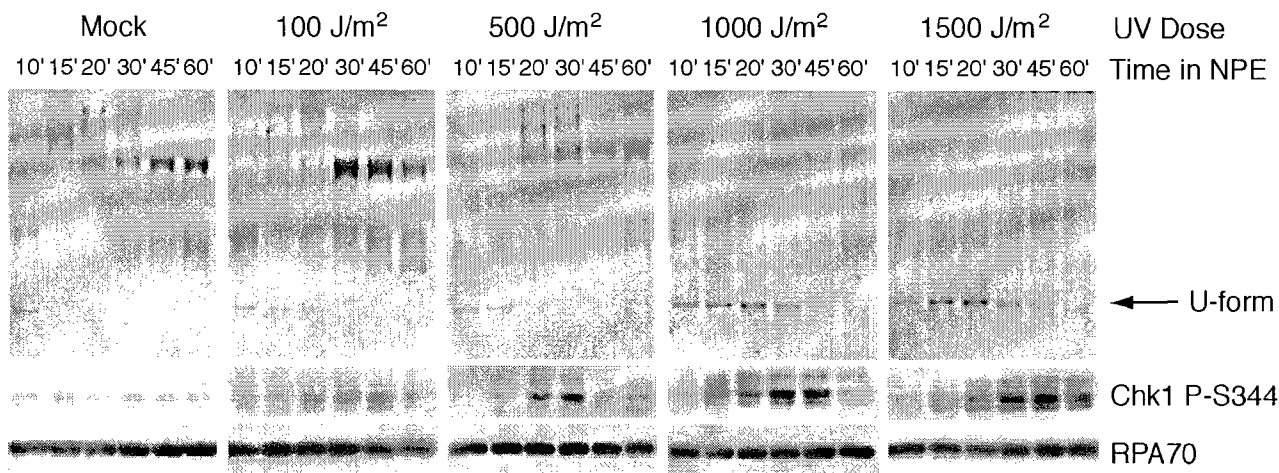
Figure 1

Figure 2

| Mock | 1.3 µM | 2.2 µM | 4.3 µM | 13.0 µM | [Aphidicolin] |
| 10'20'30'45'60'90' | 10'20'30'45'60'90' | 10'20'30'45'60'90' | 10'20'30'45'60'90' | 10'20'30'45'60'90' | Time in NPE |

← U-form

Chk1 P-S344

RPA70

Figure 3

A

Mock	100 J/m²	500 J/m²	1000 J/m²	1500 J/m²	UV Dose
10' 15' 20' 30' 45' 60'	10' 15' 20' 30' 45' 60'	10' 15' 20' 30' 45' 60'	10' 15' 20' 30' 45' 60'	10' 15' 20' 30' 45' 60'	Time in NPE

← U-form

Chk1 P-S344

RPA70

B

	Mock		Cis-Platinum		
	−	+	−	+	Geminin

Chk1 P-S344

Chk1

C

	Mock								Cis-Platinum								
	−	−	−	−	+	+	+	+	−	−	−	−	+	+	+	+	Geminin
	0'	15'	30'	45'	0'	15'	30'	45'	0'	15'	30'	45'	0'	15'	30'	45'	Time in NPE

← U-form

Figure 4

A

Sperm chromatin + cytosol
↓ 30 min
NPE
↓ 25 min
KIP
↓ 15 min
Isolate chromatin

				rCdc45 +
ELB	ELB	ELB	αCdc45	αCdc45
↓	↓	↓	↓	↓

NPE   NPE^{aph}

MCM7
ORC2
Cdc45

RPA34

Chk1

Chk1 P-S344

-	-	+	-	-	caffeine
1	2	3	4	5	

B

Sperm chromatin + cytosol
↓ 30 min
NPE
↓ 25 min
KIP
↓ 15 min
Isolate chromatin

				rMCM7 +
ELB	ELB	ELB	Rb$^{1-400}$	Rb$^{1-400}$
↓	↓	↓	↓	↓

NPE   NPE^{aph}

MCM7
ORC2
Cdc45

RPA34

Chk1

Chk1 P-S344

-	-	+	-	-	caffeine
1	2	3	4	5	

19

A

B

	800 bp plasmid	9 kB plasmid		
	1	0.5	0.1	Molar Equivalents
	1	5.6	1.1	Mass Equivalents

Chk1 P-S344

Chk1

| - | + | + | + | - | + | + | + | + | + | + | Aphidicolin |
| 90' | 30' | 60' | 90' | 90' | 30' | 60' | 90' | 30' | 60' | 90' | Time in NPE |

Figure 5

Figure 6

A

| | 0 | 15 | 150 | 375 | 1100 | Aphidicolin [µM] |

15' 30' 45' 60' 90' 15' 30' 45' 60' 90'   15' 30' 45' 60' 90' 15' 30' 45' 60' 90'   15' 30' 45' 60' 90'   Time in NPE

←——U-form

32P-dCTP
incorporation

Chk1 P-S344

Chk1

B

| 15 | 1500 | 1500 added at 25' | Aphidicolin [µM] |

10' 20' 30' 40' 60' 80' 100'   10' 20' 30' 40' 60' 80' 100'   10' 20' 30' 40' 60' 80' 100'   Time in NPE

Chk1 P-S344

Chk1

C

SJK-132

| Untreated | 44 µg/ml | 176 µg/ml | 880 µg/ml | Aphidicolin |

40' 60' 80' 100'   40' 60' 80' 100'   40' 60' 80' 100'   40' 60' 80' 100'   40' 60' 80' 100'

Chk1 P-S344

RPA70

21

Figure 7

0   25   740   Aphidicolin [μM]

Chromatin Bound

Polα

RPA70

ATRIP

Rad1

PCNA

Soluble

Chk1 P-S344

Chk1
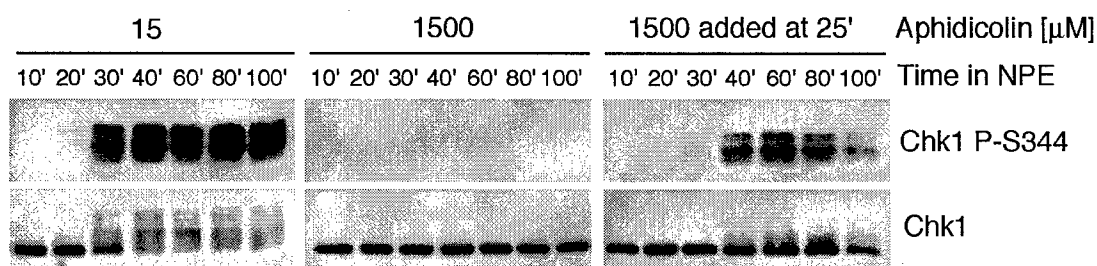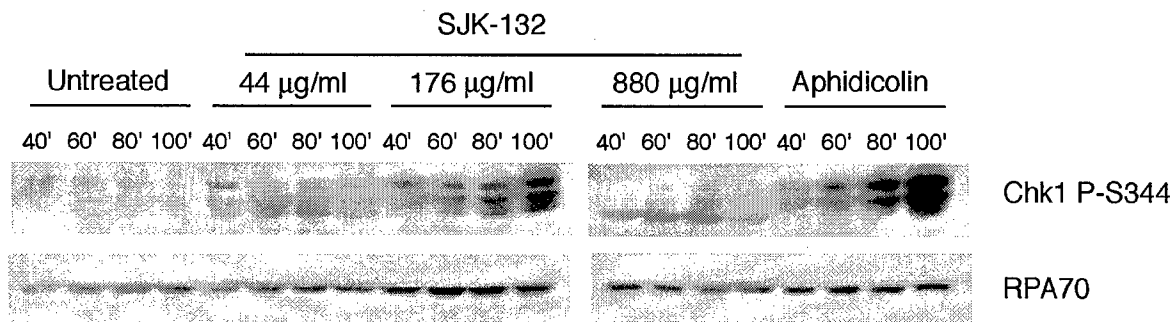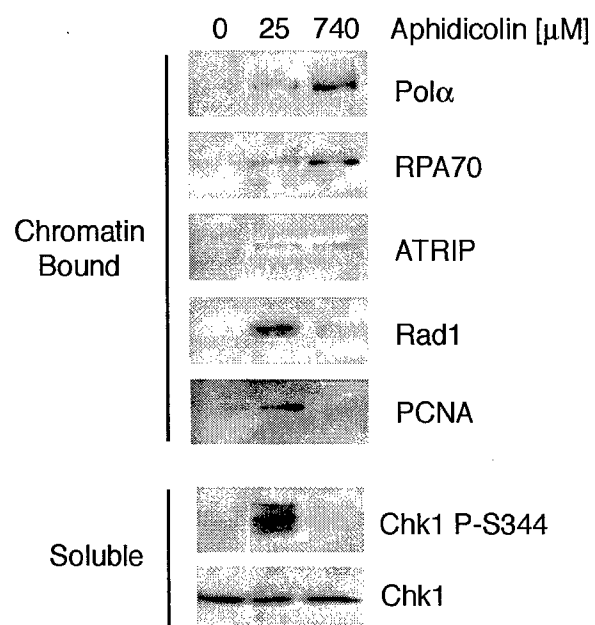
Key Research Accomplishments

Demonstrated that a plasmid-based system could be used to study the ATR-dependent checkpoint pathway.

Demonstrated that replication of damaged DNA results in DNA hyperunwinding.

Demonstrated that hyperunwinding of DNA is required for checkpoint activation.

Demonstrated that the level of checkpoint activation is proportional to the amount of unwound DNA.

Demonstrated that DNA synthesis is required for activation of the ATR checkpoint pathway.

Reportable Outcomes:

Publications:

Tony S. Byun, Marcin Pacek, Muh-ching Yee, Johannes C. Walter, and Karlene A. Cimprich (2005) Functional uncoupling of MCM helicase and DNA polymerase activities activates the ATR-dependent checkpoint. *Genes Dev.* In Press

Degrees:
Tony Byun obtained his PhD in March 2005 while being supported by the DOD fellowship.

Employment:
Tony Byun obtained a Staff Scientist position at Catalyst Biosciences based on research experience obtained during his DOD research fellowship.

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